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Simplified fast and high yielding automated synthesis of [^{18}F]fluoroethylcholine for prostate cancer imaging

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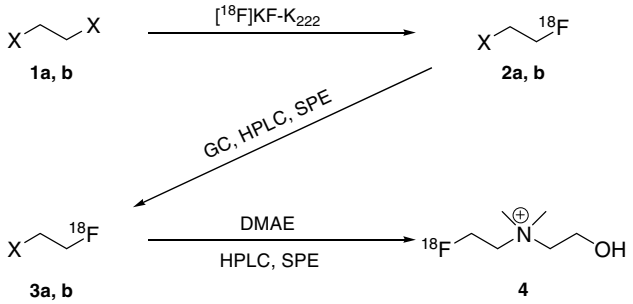
ABSTRACT

^{11}C- and ^{18}F-labeled choline analogues are successful tracers for prostate cancer imaging using positron emission tomography (PET). Due to the practical advantages of the longer-living radioisotope ^{18}F ($t_{1/2}$ = 110 min) instead of ^{11}C ($t_{1/2}$ = 20 min), [^{18}F]fluoroethylcholine has been introduced to increase the opportunity of widespread clinical application. Nevertheless, the various known synthetic methods provide [^{18}F]fluoroethylcholine for human use only in moderate overall yields of up to 30% so far.

Here, a new simplified and high yield two-step-synthesis for [^{18}F]fluoroethylcholine is described for potential clinical applications starting from 2-bromoethyl triflate (BETfO) using a modified, commercially available fully automated synthesis module. All synthesis parameters were subsequently optimized resulting in a total yield of 47 ± 5% (not decay corrected) in only 40 min. [^{18}F]fluoroethylcholine could be obtained ready for human use as physiological solution after fixation on Sep-Pak Accell Light cartridges (waters®) and formulation with saline without the need of GC or HPLC purification. Radiochemical purity was >99.9% and no contamination of the sterile solution with chemicals used during the synthesis was detected.

1. Introduction

One essential aspect in biological properties of cancer cells is their uncontrolled growth. This leads to a significant increase in the synthesis of phosphor lipid membranes that is associated with choline transporter and choline kinase enzyme overexpression.[1,2] Due to an elevated uptake and phosphorylation of choline to form choline phosphate, in many human cancers the intracellular concentration of phosphocholine is well above the normal level.[3,4] Therefore, [^{11}C]choline was introduced as a very effective tracer in the positron emission tomography (PET) for imaging various human tumors.[5,6] Because of the short half-life time of ^{11}C (20 min) ^{18}F labeled ($t_{1/2}$ = 110 min) choline analogues have been developed to increase the possibilities of a widespread use in routine diagnostics. The first approach to [^{18}F]fluorocholine ([^{18}F]FC) was reported by DeGrado et al.[7] At that time it was synthesized by reacting *N*,*N*-dimethylaminoethanol (DMAE) with bromo-[^{18}F]fluoromethane ([^{18}F]BFM). Since this method required a time consuming gas chromatographic purification of [^{18}F]BFM during the synthesis,[8] various other strategies have been introduced for the synthesis of ^{18}F labeled compounds via ^{18}F-fluoroethylation.[8–12] The most successful used common syntheses for [^{18}F]fluoroethylcholine ([^{18}F]FECh **4**) are demonstrated in scheme 1. Starting from precursor 1,2-dibromoethane (DBE **1a**) or 1,2-bis(tosyloxy)ethane (DITOS **1b**) the ^{18}F-labeled ethyl derivatives (prosthetic groups **2a, b**) are synthesized in a first reaction step by a nucleophilic substitution. In the case of DBE **1a** (bp 131 °C) the intermediate 1-bromo-2-[^{18}F]fluoroethane ([^{18}F]BFE **2a)** (bp 71.5 °C)[13] has to be separated via gas chromatography before the final reaction with DMAE can be performed leading to [^{18}F]FECh **4**. Even if DITOS **1b** is used, it is neces-



a: X = Br, **b**: X = OTs

Scheme 1. Common syntheses of [^{18}F]fluoroethylcholine.

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sary to introduce an additional purification step either before starting the second reaction via SPE/HPLC[8] or a final separation using HPLC.[13,14] Therefore, both methods require considerable efforts concerning the purification and even more important consume a lot of precious time. That is why only low yields of [^{18}F]FECh could be achieved until now. Thus, the obtainable yield of a fully automated synthesis of [^{18}F]FECh is limited to only 30% and lasts as long as 50 min.[14] For an application in clinical services this means a relative high dose cost rate, which certainly limits a widespread use of [^{18}F]FECh.

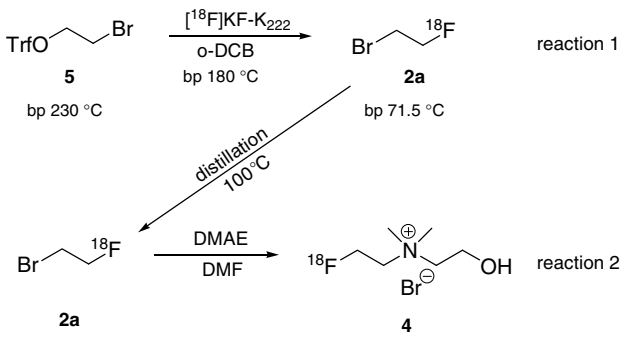
Thus, our aim was to develop a simplified and considerably faster automated synthesis with higher yields making this approach become much more attractive for clinical routine use. In this paper, we are describing the development of a new protocol for the synthesis of [^{18}F]FECh via ^{18}F-fluoroethylation using 2-bromoethyl triflate (BETfO **5**) as the starting material in a fully automated fast synthesis without the need of GC or HPLC purification.

2. Results and discussion

BETfO **5** was subjected to nucleophilic substitution with the [^{18}F]KF-Kryptofix®-complex ([^{18}F]KF–K_{222}) in 1,2-dichlorobenzene (*o*-DCB) (Scheme 2).

The advantage of BETfO **5** compared to DBE **1a** is not only the higher reactivity of its triflate group compared to the bromine of DBE but especially its higher boiling point (230 °C) that is around 160 °C higher than the boiling point of [^{18}F]BFE **2a** (bp 71.5 °C). Thus, in contrast to DBE the use of less volatile BETfO **5** enables a simple distillation of the intermediate [^{18}F]BFE **2a** leaving all impurities from the first reaction behind in reactor 1. In this way, the second reaction of [^{18}F]BFE **2a** with DMAE can be easily performed under pure conditions resulting in higher specific activity of [^{18}F]FECh **4**.

We then turned our attention to the individual optimization of the three processes (reaction 1, distillation and reaction 2) outlined in Scheme 2.



Scheme 2. Two pot synthesis of [^{18}F]fluoroethylcholine.

2.1. Reaction 1: Formation of [^{18}F]BFE 2a

The adequate formation of the [^{18}F]KF–K_{222}-complex is essential for the fluorination reaction of BETfO **5** leading to [^{18}F]BFE **2a**. Therefore, the effect of the used amounts of the reactants, K_2CO_3/K_{222}, and BETfO **5**, as well as the effect of the drying temperature and the helium flow rate on the formation of [^{18}F]BFE **2a** was studied. The results are summarized in Figure 1. The maximum yield of [^{18}F]BFE **2a** was achieved when using 80 μmol K_2CO_3/K_{222} and 30 μL BETfO **5**. The study of the effect of the helium flow rate and drying temperature indicated an optimum of 100 mL/min and 90 °C, respectively. At higher temperatures the yield of [^{18}F]BFE **2a** became lower due to decomposition of [^{18}F]KF–K_{222}.

2.2. Distillation of [^{18}F]BFE 2a

A simple distillation at 130 °C under helium flow transfers the intermediate [^{18}F]BFE **2a** in almost pure form into the second reactor containing DMAE in DMF. The dependence of the portion of the intermediate transferred through the distillation on the temperature and the helium flow rate is shown in Figure 2. It turned out that the maximum yield was obtained when a flow rate of 100 mL/min and a temperature of 130 °C were applied. Higher temperatures led to less transferred material because of partial decomposition of BETfO **5**. Gas chromatographic analysis of the distilled material showed no presence of BETfO **5** (bp 230 °C) in the second reactor when the temperature was kept ⩽130 °C.

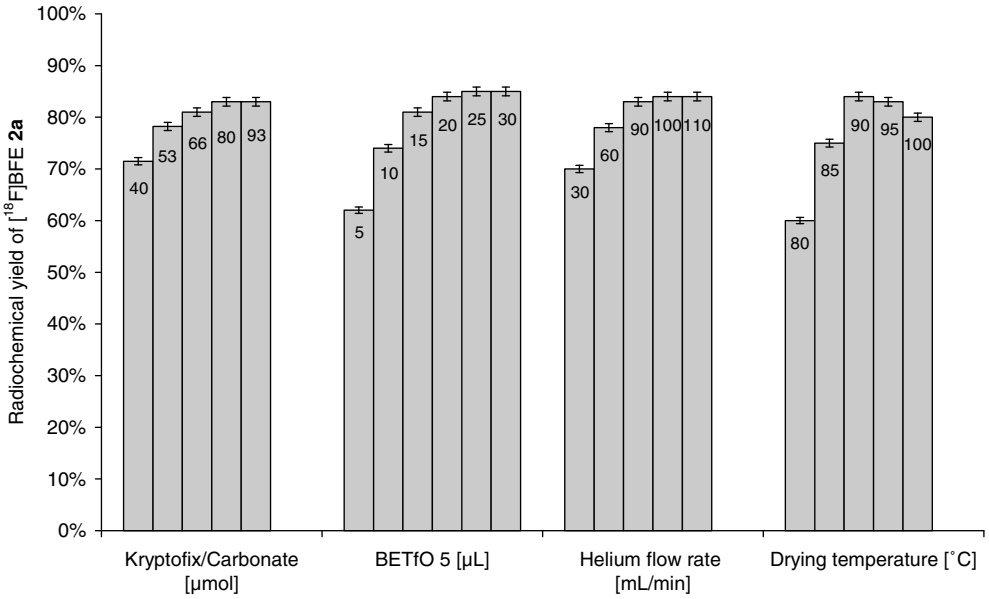


Figure 1. Dependence of the radiochemical yield of [^{18}F]BFE **2a** on the amount of reactants, drying temperature of [^{18}F]KF–K_{222} and helium flow (n = 3).

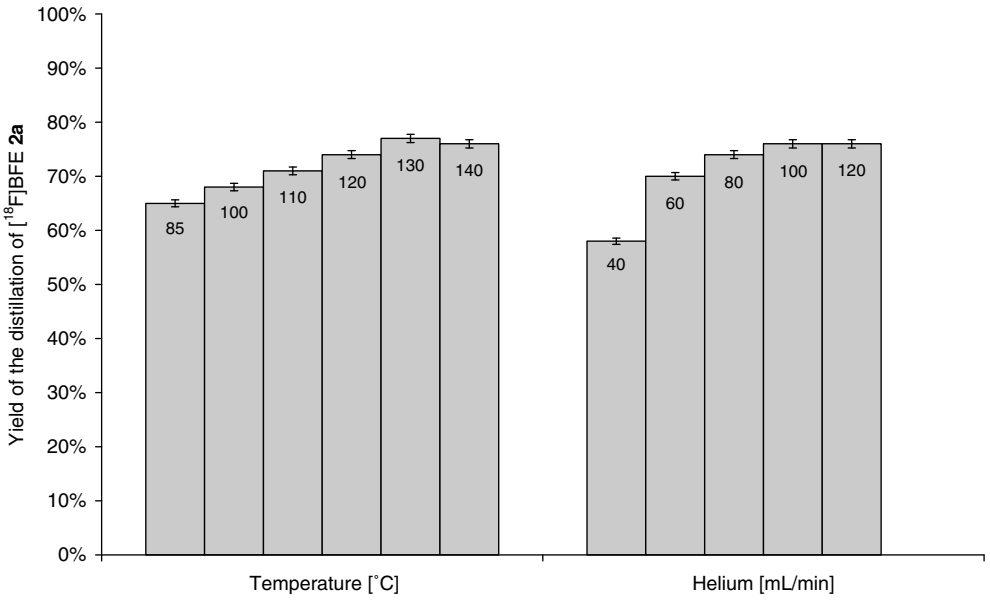


Figure 2. Dependence of the yield of distillation of [^{18}F]BFE **2a** on temperature and helium flow ($n = 3$).

2.3. Reaction 2: Formation of [^{18}F]FECh (4)

The second reaction between [^{18}F]BFE **2a** and DMAE was carried out in the second reactor in DMF. In order to optimize the radiochemical yield of [^{18}F]FECh (**4**) the effect of reaction time and reaction temperature as well as the effect of the amount of DMAE and DMF were subsequently studied. These results are shown in Figure 3. 0.5 mL of DMAE in 1.7 mL of DMF was found to be the optimum concentration in order to obtain the highest yield in reaction 2 (Fig. 3). The optimization of the reaction temperature and reaction time resulted in a maximum yield obtained at 100 °C and 15 min, respectively.

2.4. Chemical and radiochemical purity

All non-volatile reactants and products of reaction 1, K_{222}, K_2CO_3 and [^{18}F]KF–K_{222} stay behind in reactor 1 and only [^{18}F]BFE **2a** is transferred to reactor 2 upon distillation at 130 °C. Thus, they do not interfere with the second reaction. The only compounds that might possibly be also transferred into reactor 2 would be *o*-DCB (bp 180 °C) and BETfO **5** (bp 230 °C) due to their considerable vapour-pressure. GC measurements of the distillate, however, clearly revealed that only a trace amount of *o*-DCB (5 μL/mL) is actually present in the distillate whereas BETfO **5** was not detected at all (detection limit <0.01 ng/mL). This is an important finding because BETfO **5** could also react with DMAE to give, for example, 2-bromoethylcholine which would cause problems in the following separation of [^{18}F]FECh **4** via the cationic exchange cartridges. The contamination with *o*-DCB, however, could easily be removed together with DMF and DMAE by diluting the reaction mixture with ethanol, loading onto the Sep-Pak cartridges, and subsequent cleaning with ethanol and water. As demonstrated in Figure 4a and Table 1 the investigation of the final sterile saline solution of [^{18}F]FECh via gas chromatography showed

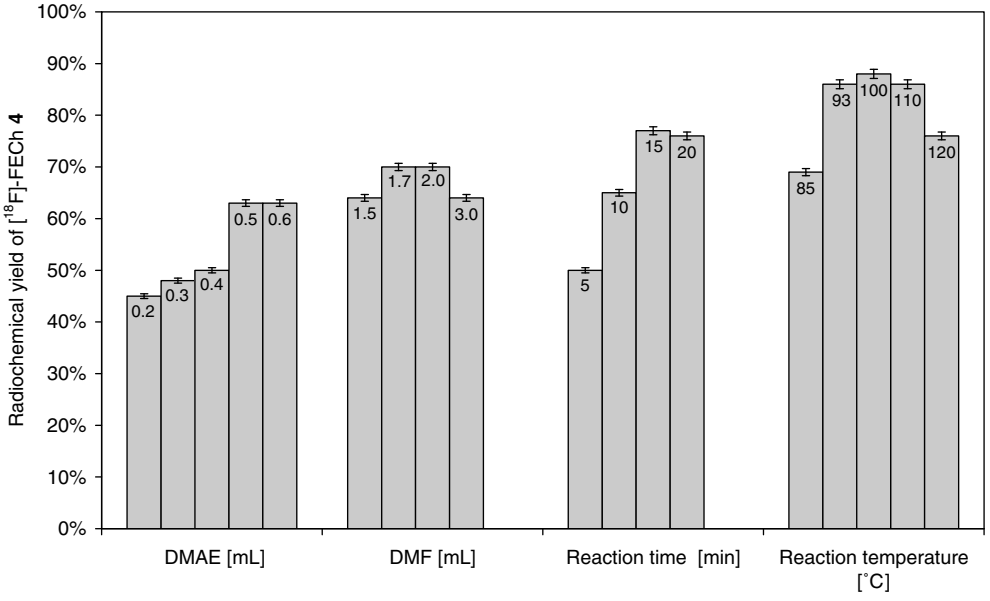


Figure 3. Dependence of the radiochemical yield of [^{18}F]FECh **4** on the quantity of DMAE and DMF as well as on reaction time and temperature ($n = 3$).

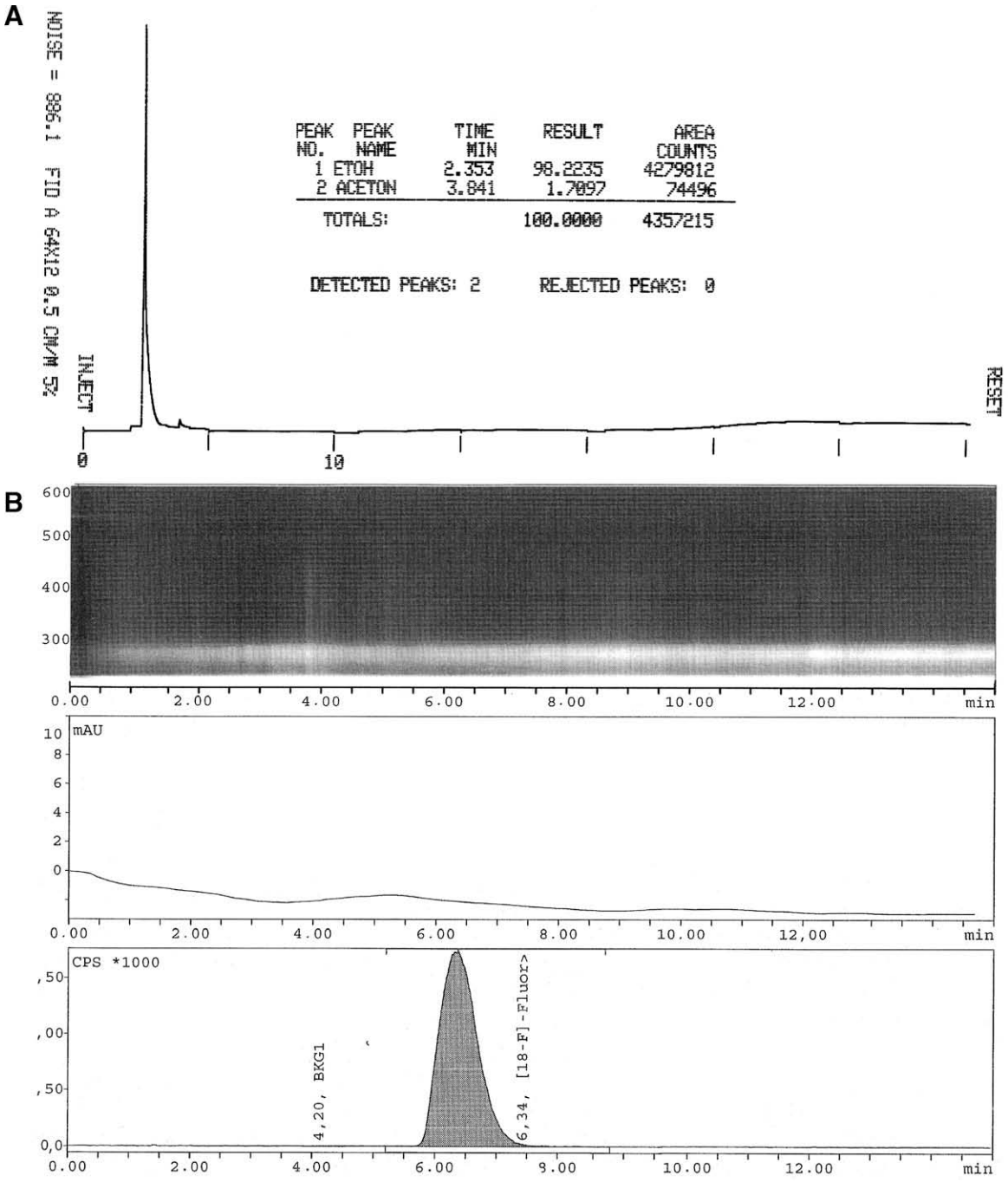


Figure 4. Chemical and radiochemical purity detection with (A) gas chromatography, (B) radio HPLC with diode array detection.

Table 1
Chemical purity of the final ready for humane use solution of [^{18}F]FECh **4** in saline detected with the gas chromatography

Solvent	Acetone	Ethanol	*o*-DCB	DMF	DMAE
[ng/mL]	0.24	11.5	—	—	—
det. limit [ng/mL]	0.007	0.007	0.002	0.007	0.025

only the present of minimal amounts of acetone from the cleaning procedure (0.24 ng/mL) and ethanol (11.5 ng/mL). No other solvents could be detected (Table 1).

The purity and identity were checked by ion exchange chromatography (Partisil 10 SCX 250 4.6 mm, 0.25 M H_3PO_4/KCl, 10%V acetonitrile, flow rate: 1.0 mL/min). The radiochemical yield was 47 ± 5% with a radiochemical purity of >99.9% and a specific activity >55 GBq/μmol. Typical chromatograms of the quality control are shown in Figure 4.

3. Conclusion

Due to the use of non-volatile 2-bromoethyl triflate as the starting material and the introduction of a distillation step instead of an HPLC purification we were able to significantly simplify, shorten, and improve the synthesis of [^{18}F]fluoroethylcholine. At the same time we could increase the radiochemical yield and the chemical purity dramatically. Now, the radiochemical yield of [^{18}F]fluoroethylcholine of 47 ± 5% is almost comparable with the radiochemical yield of [^{18}F]FDG. Thus, our optimized and easy to handle

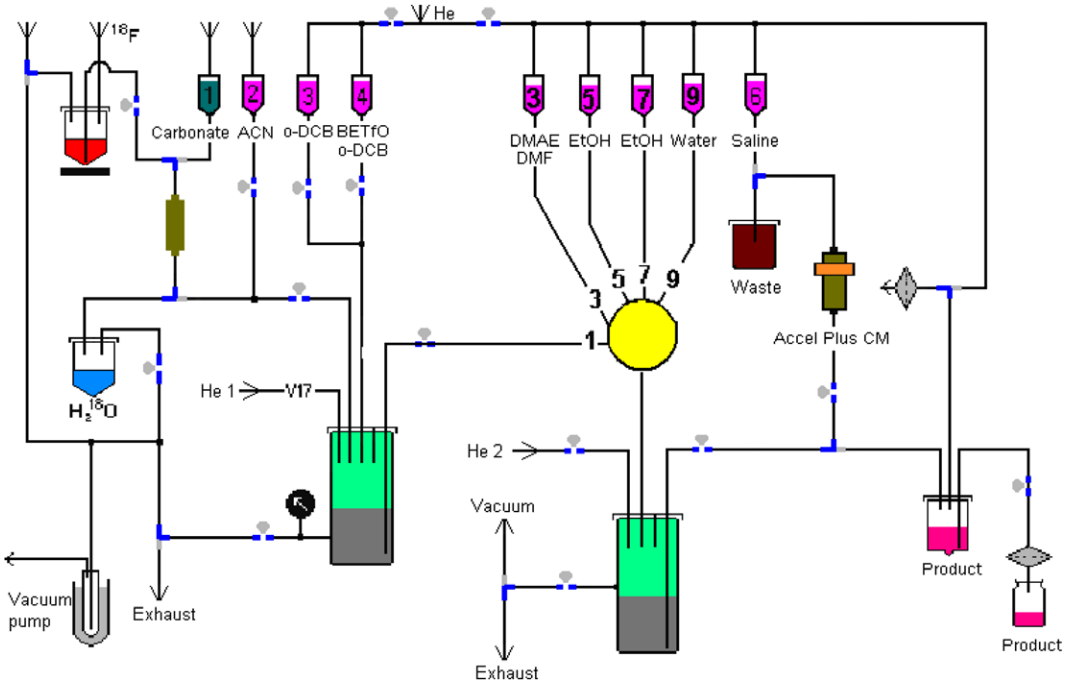


Figure 5. Schematic diagram of the automated synthesis module.

synthesis provides access to this important compound for routine clinical use as a radio diagnosticum.

4. Experimental

4.1. Materials and methods

Chemicals were purchased from commercial sources and were used without further purification. The Sep-Pak® Light Accell Plus CM cartridges were purchased from Waters, [^{18}F]fluoride was produced via the ^{18}O(p,n)^{18}F reaction with a CTI RDS 111 cyclotron (Berlin). HPLC was performed with HP 1100 pump with UV and gamma radiation detection (Gabi, Raytest). All demonstrated data are not decay corrected. Gas chromatography was performed with Varian 3350 spectrometer and FID detection Figure 4A.

4.2. 2-Bromoethyl triflate BETfO 5

BETfO **5** was synthesized as described in the literature.[15] 2-Bromoethanol (1 g, 8.0 mmol) was dissolved in 2.05 mL of 2,6-lutidine (17.6 mmol), diluted with 10 mL of CH_2Cl_2 and cooled to 0 °C. Trifluoromethanesulfonic acid anhydride (2.83 mL, 16.8 mmol) was added dropwise. After being stirred for 30 min, the solvent was removed in vacuum and the product was distilled (50 °C at 0.5 mm Hg) to give 3.5 g (53%) of **5** as a colorless liquid: GC chromatogram, single peak, t_R = 7.82 min (column Rtx 200, 0.25 mmID, 30 m, carrier gas N_2, 0.95 mL/min, injector 250 °C, program 50 °C (5 min hold, than 10 °C/min).

4.3. Radiosynthesis

The synthesis of [^{18}F]FECh **4** was carried out in the style of GMP. [^{18}F]FECh **4** was produced in a hot cell equipped with a commercially available automated synthesis module using sterile filtered helium as gas carrier (Fig. 5). All starting materials were tested using validated methods and qualified equipment. The quality control involved measurement of the chemical and the radiochemical purity via HPLC, gas chromatography and the pH and other parameters were tested.

[^{18}F]Fluoride ion (2–10 GBq) obtained from the target was trapped in a small anion exchange column (30 mg, HCO_3 form). The radioactivity was eluted with an aqueous solution of K_2CO_3 (11 mg; 0.08 mmol) in 0.8 mL of H_2O into reactor 1. A solution of K_{222} (30 mg; 0.08 mmol) in 1.5 mL of CH_3CN was added, and the mixture was evaporated at 90 °C under reduced pressure with a helium flow of 100 mL/min for 2 min and without helium flow for additional 2 min. 0.5 mL of *o*-DCB were then added to the dried [^{18}F]KF–K_{222}. After one minute of stirring at 100 °C BETfO **5** (25 μL) dissolved in 0.5 mL of *o*-DCB was added and the temperature was allowed to increase to 130 °C while [^{18}F]BFE **2a** was transferred within 5 min into the second reactor containing 0.5 mL of DMAE (5.0 mmol) in 1.7 mL of DMF. The second reactor was then tempered for 15 min at 100 °C. Afterwards the temperature was decreased to 60 °C. Five milliliters of ethanol was added and the reaction mixture was passed through a Sep-Pak Accell Light cartridge (Waters). The cartridge was then washed with 10 mL of ethanol and 10 mL of H_2O, respectively. The [^{18}F]FECh **4** was then eluated with 1 mL of 10% aqueous NaCl and diluted with 9.0 mL of sterile water. After sterile filtration 0.94 to 4.7 ± 5% GBq [^{18}F]FECh **4** were obtained. The radiochemical purity was >99.9% and the specific activity >55 GBq/μmol.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.09.031.

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